Defective axonal transport in motor neuron disease

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Axonal transport and motor neuron disease

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Abstract:

A number of recent studies have highlighted the role of axonal transport in the pathogenesis of motor neuron diseases. Mutations in genes that control microtubule regulation and dynamics have been shown to cause motor neuron degeneration in mice and in a form of human motor neuron disease. In addition, mutations in the molecular motors dynein and kinesins and several proteins associated with the membranes of intracellular vesicles that undergo transport cause motor neuron degeneration in humans and mice. Paradoxically, evidence from studies on the legs at odd angles mouse (*Loa*) and a transgenic mouse model for human motor neuron disease suggest that partial limitation of the function of dynein may in fact lead to improved axonal transport in the transgenic mouse leading to delayed disease onset and increased life span.

Key words:

Motor neuron disease, dynein, kinesin, legs at odd angles, *Loa*, Cramping 1, *Cra1*, superoxide dismutase-1, SOD1

AXONAL TRANSPORT IN MOTOR NEURONS

Motor neurons are highly specialised cells with volumes of up to 5,000 times larger than the volume of an average cell. Their axons can be more than a meter long in the humans and each motor neuron forms many synapses with other neurons and muscle cells. These properties of motor neurons and their high energy throughput necessitate a highly efficient and finely tuned transport system for the delivery of organelles, such as mitochondria and endosomes, as well as structural and signalling proteins into the axons and for transporting neurotrophic factors from the axon termini to the perikaryon. The major components of this transport system are a group of specialised motor proteins plus the cytoskeletal networks of microtubules and actin filaments. Motor proteins use these networks as intracellular tracks for transporting their cargos.

As part of their cytoskeleton neurons also contain neurofilaments which are comprised of the neurofilament triplet proteins neurofilament light chain (NF-L), middle chain (NF-M), and heavy chain (NF-H). Other neuronal intermediate filaments include α-internexin and peripherin. Neurofilaments are the major intermediate filaments in most terminally differentiated neurones whereas α-internexin is expressed more abundantly in the developing nervous system (Fliegner et al. 1994; Kaplan et al. 1990), and peripherin is expressed mostly in autonomic nerves and sensory neurons (Escurat et al. 1990; Parysek and Goldman 1988; Troy et al. 1990). The speed of conductivity of an impulse down the axon is in part proportional to its calibre and an important function of neurofilaments is to control axonal calibre. Thus

neurofilaments are particularly abundant in neurones with large-diameter axons including those of motor neurones where fast impulse conduction velocities are crucial for proper functioning.

Actin filaments are highly enriched in the cell cortex, whereas microtubules and neurofilaments run the length of the neurons. Of these the actin filaments and microtubules are directly involved in axonal transport. Microtubules are long filaments formed by the polymerisation of tubulin dimers consisting of one α- and one β- tubulin polypeptides. The α- and β- tubulins are members of the same protein family with up to 40% sequence homology and they are also highly conserved across species (Cleveland and Sullivan 1985). The regular orientation of these tubulin heterodimers confers a structural polarity to the microtubules so that one end – the plus end – is the preferred end for addition of tubulin dimers whereas the other end – the minus end – grows more slowly under physiological concentrations of tubulin. A third member of the tubulin family is γ-tubulin which is found at microtubule organising centres (MTOC) and it is thought to be involved in nucleation of microtubules and anchoring the minus-end (Joshi 1993).

In the axons microtubules form long, but not continuous, filaments which have a uniform polarity with the plus-end directed away from the cell body, whereas in the dentrites they are generally shorter and they are non-uniform with respect to polarity (Baas et al. 1988). As a result of their association with various microtubule associated-proteins (MAPs), microtubules in the axons and dendrites are much more stable than those in most interphase cells. In

addition, MAPs contribute to the composition of a network of filamentous structures that cross-link microtubules in the neurites and owing to the difference between the composition of the MAPs in axons and dendrites the spacing between microtubules in axons (~20 nm) differs from that in the dendrites (~65 nm) (Hirokawa 1982; Hirokawa and Takemura 2005). This is achieved partly by selective transport and stabilisation of tau (a microtubule associated protein) in axons and MAP2 in dendrites (Hirokawa et al. 1996; Kanai and Hirokawa 1995; Okabe and Hirokawa 1989). Moreover, microtubule dynamics and remodelling in neurons are crucial for the extension, arborisation, and guidance of the axons. These processes are regulated by the functions of MAPs and other enzymes that take part in the stabilisation and breakdown of the microtubules (Siegel 1999).

Cytoplasmic dynein, kinesin, and unconventional myosin families are the molecular motors that use microtubules and actin filaments as tracks for intracellular transport within neurons. These motors are structurally similar and they all have a globular domain (the head) which forms their motor unit and a rod shaped domain (the tail) involved in cargo binding. The head domains in dynein and kinesins contain microtubule binding domains. Myosins on the other hand have actin binding domains - although there is evidence that some unconventional myosins interact with microtubules too (Cao et al. 2004; Weber et al. 2004). It is through these domains that the motor proteins bind to microtubules or actin filaments and by hydrolysing ATP generate energy to slide along these tracks.

Miki et al identified 45 genes that belong to the kinesin superfamily in mice and humans (Miki et al. 2001). They classified the 45 kinesins into three types, based on the position of the motor domain in the protein, as amino terminal motor (N-kinesins), middle motor (M-kinesins), and carboxy terminal motor (C-kinesins) types. The motor domain in the kinesin superfamily is highly conserved but there is significant divergence in their cargo binding domains, conferring diverse binding properties for binding of a wide range of cargo. In contrast to kinesins, cytoplasmic dynein is encoded by two genes with *DNCHC1* coding for the majority of the cytoplasmic dynein in neurons. The cargo binding diversity of cytoplasmic dynein comes about by the heterogeneity of the auxiliary subunits that bind to the tail of this protein.

The net directions of dynein and kinesin movements are defined by the plus/minus end polarity of microtubules. N-kinesins and M-kinesins generally move towards the plus end and C-kinesins appear to move towards the minus end of the microtubules (Hirokawa and Takemura 2005). Cytoplasmic dynein on the other hand is classified as a motor protein that moves towards the minus-end. Although Ross *et al* have recently shown that this protein indeed moves in both directions along the microtubules (Ross et al. 2006). Their study revealed that 30% of the processive runs of single dynein-dynactin complexes were directed towards the microtubule plus ends. However, the net movements of dynein-dynactin complexes were towards the minus-end in both frequency and run length (Ross et al. 2006).

Like microtubules actin filaments are also polar with the plus end being more dynamic. But unlike microtubules, actin filaments are randomly orientated and form shorter filaments. Unconventional myosins such as Myosin V bind to these filaments and move toward the plus end.

AXONAL TRANSPORT AND MOTOR NEURON DISEASE

Motor neuron diseases (MND) constitute several groups of heterogeneous disorders that affect adults as well as children. These disorders include spinal muscular atrophy (SMA), hereditary spastic paraplegias (HSPs), and amyotrophic lateral sclerosis (ALS). Below we will describe the current understanding of the disease mechanisms in some of these disorders in relation to the axonal transport system (summarised in table 1 and figure 1). We will first discuss motor neuron diseases that are caused by impairment of microtubule dynamics and compromised vesicle trafficking, then we will address mutations in motor proteins, which lead to motor neuron degeneration. Finally, we will discuss the extensively researched mutant superoxide dismutase 1 (SOD1) that causes familial ALS, focusing on the impairment of the axonal transport as an important factor in the pathology of ALS.

Microtubule dynamics and MND

As mentioned above a highly efficient microtubule mediated communication system is essential for the proper functioning and health of the motor neurons and thus any compromise in the microtubule assembly and dynamics could have deleterious consequences for these cells. This has been highlighted by

the identification of the causal mutation in the *progressive motor neuropathy (pmn)* mouse. The *pmn* is an autosomal recessive trait in the mouse, which is characterised by progressive caudio-cranial degeneration of the motor axons from two-weeks of age followed by death by the age of 4 – 6 weeks. Phenotypically *pmn* resembles the recessive proximal childhood form of spinal muscular atrophy (SMA). A missense point mutation, resulting in a W524G substitution, in the gene encoding the *tubulin-specific chaperone E* (*Tbce*) has been shown to be responsible for *pmn* (Bommel et al. 2002; Martin et al. 2002). The TBCE protein appears to be ubiquitously expressed in the nervous system and other organs, with no selectivity for the spinal cord (Bommel et al. 2002). It binds to α-tubulin and mediates the assembly of α–β heterodimers in the tubulin folding pathway (figure 1). In *pmn* mice, however, the W524G mutation seems to make the protein significantly less stable than its wild-type counterpart (Martin et al. 2002). The molecular mechanisms of motor neuron degeneration in *pmn* are not fully understood yet, but the selective vulnerability of motor neurons in *pmn* mice may reflect the highly sensitive nature of this group of neurons to any disturbance in the components of the axonal transport system.

Impaired microtubule dynamics has also been implicated in a form of hereditary spastic paraplegia (SPG4). The HSPs are a clinically and genetically heterogeneous group of motor neuron diseases that are characterised by progressive lower limb spastic paralysis. This group of diseases are conventionally divided into pure and complicated subgroups based on absence or presence of additional neurological or non-neurological

clinical features (Reid 2003). Pure HSPs are the most common forms of the disease and they are histopathologically characterized by the “dying back” of the terminal ends of the corticospinal tract axons, usually with longest axon being involved first. At least 20 genes are thought to be involved in HSPs and of those the most important gene in empidemiolgical terms is SPG4. Mutations in SPG4 cause an autosomal dominant form of the disease and they are responsible for approximately 40-50% of pure HSPs. The SPG4 gene product is the microtubule interacting protein spastin (Hazan et al. 1999). Spastin is ubiquitously expressed in all tissues but with slightly higher expression in the foetal brain and it is thought to be a microtubule severing protein involved in axon development and maintenance (figure 1).

In their analysis of the role of spastin in neuronal development using zebrafish embyo as a model system Wood et al have shown that reducing the function of the zebrafish ortholog of spastin by antisense oligonucleotides significantly impairs motor axon outgrowth and enhances apoptosis throughout the CNS (Wood et al. 2006). They also observed abnormal neuronal connectivity throughout the spinal cord (Wood et al. 2006). Mutations in spastin typically cause adult-onset HSP with the age of onset being widely variable both within and among families and evidence suggest that haploinsufficiency is a likely pathogenic mechanism in this disease (Burger et al. 2000). However, all reported human HSPs with mutations in both spastin alleles (compound heterozygotes) have infantile or childhood onset. Data from wood et al suggest that a 50% decrease in spastin function is sufficient for near normal neuronal development but it would be detrimental in long term axonal

maintenance. It is therefore plausible to suggest that the presence of only one normal allele leads to late-onset motor neuron disease, whereas less than 50% spastin activity would result in neurodevelopmental defects causing childhood disease (Wood et al. 2006).

In a series of yeast-two-hybrid assays using spastin as bait, Reid and Sanderson et al identified several proteins that interact with spastin, including CHMP1B and atlastin (Reid et al. 2005; Sanderson et al. 2006). CHMP1B is a component of the ESCRT (endosomal sorting complex required for transport)-III complex which is responsible for vesicle budding, leading to the release of a vesicle carrying specific membrane cargo into the interior of the endosomes. Atlastin on the other hand is a GTPase protein and evidence suggests that it is localised in the Golgi and/or ER membrane (Sanderson et al. 2006). Interestingly, mutations in Atlastin have been shown to cause SPG3, another form of hereditary spastic paraplegia (Zhao et al. 2001) – see below. In addition, Evans et al have independently reported the interaction between spastin and atlastin and they have shown that a clinical mutation in atlastin prevents its interaction with spastin (Evans et al. 2006).

Given the role of spastin in microtubule regulation and its interaction with CHMP1B and atlastin it is possible that spastin has at least two distinct but related roles in neurons. One is the regulation of the microtubule dynamics and its other role is to be recruited by adaptor membrane proteins to regulate membrane trafficking (Sanderson et al. 2006) (figure 1).

Membrane-associated proteins and MND

Atlastin is most abundant in the brain and spinal cord and mutations in this gene are responsible for >10% of autosomal dominant pure HSPs and to date all reported mutations in this gene show childhood onset (Muglia et al. 2002; Zhao et al. 2001). How these mutations affect the function of atlastin and how they cause HSP is not clear yet, but based on the sequence homology of this protein with dynamins it is possible that like dynamins, atlastin is involved in vesicle trafficking events and it may have a role in the distribution and maintenance of the mitochondria (Zhao et al. 2001). In addition, spartin the gene implicated in Troyer syndrome, which is an autosomal recessive HSP, has been shown to localise to mitochondria and interact with microtubules (Benashski et al. 1997; Proukakis et al. 2004; Teuchert et al. 2006). Thus it is likely that mutations in spartin impair microtubule-mediated trafficking of mitochondria and/or mitochondrial function leading to HSP (figure 1). Troyer syndrome, like SPG3 and the homozygous or compound heterozygous SPG4, is an early childhood disease (OMIM 275900). It would therefore be interesting to see whether spartin and atlastin are in the same pathway as spastin and whether they have a role in the early development of the axons.

Recently mutations in CHMP2B which like CHMP1B is a component of the ESCRT complexes have been reported in two unrelated patients with ALS spectrum disorders. One patient showed progressive muscular atrophy and the other showed features of ALS-dementia presenting with frontal lobe features before developing ALS (Parkinson et al. 2006). CHMP2B, CHMP1B, atlastin, and spartin provide a link between membrane traffic events, axonal

transport and motor neuron degeneration (figure 1). This link is further strengthened by the finding that a mutation in the vacular-vesicular protein sorting factor-54 (VPS54) is responsible for motor neuron degeneration in the *Wobbler* mouse (*wr*) (Duchen and Strich 1968; Schmitt-John et al. 2005). In this mouse motor neurons undergo swelling and vacuolization of cytoplasm, with cell body atrophy. The mice typically die by 3 months of age with degeneration in the cell bodies of the brainstem and spinal cord, and denervation atrophy of skeletal muscle (reviewed by Nicholson et al (Nicholson et al. 2000)). The *wr* mutation confers an autosomal recessive trait and it is in the highly conserved residue 967 changing leucine to glutamine in the carboxy-terminal region of VPS54 (Schmitt-John et al. 2005). Further analyses of this mouse revealed defective axonal transport in the motor neurons (Mitsumoto et al. 1990; Mitsumoto et al. 1993). VPS54 is a component of the Golgi-associated-retrograde protein (GARP). Other partners of VPS54 in this heterotetrameric complex in S*accharomyces cerevisiae* include VPS51, VPS52, and VPS53. It is not known how the *wr* mutation leads to motor neuron degeneration, but based on the studies in yeast, a likely explanation is that the L967Q mutation in VPS54 impairs the trafficking of the early-endosomes (Quenneville et al. 2006) (figure 1).

Perturbed endosomal trafficking has also been highlighted in an autosomal recessive juvenile form of motor neuron disease caused by mutations in *ALS2* (OMIM 205100). ALS2 is a guanine nucleotide exchange factor (GEF) which through its carboxy-terminal VPS9 domain activates the small GTPase protein Rab5. In addition, the VPS9 domain mediates the localization of ALS2 to

early-endosomal compartments (Otomo et al. 2003). Preliminary studies suggested that the Als2-null mice did not show obvious developmental, reproductive or motor abnormalities. However, Hadano et al observed these mice through 21 months of age and have recently reported an age-dependent slowly progressive loss of cerebellar Purkinje cells in these mice (Hadano et al. 2006). The Als2-null mice also exhibited lower motor neuron disturbances associated with astrocytosis and microglial cell activation indicating a sub-clinical condition in these mice (Hadano et al. 2006). In addition, these researchers provide further evidence implicating the impairment of the endosomal fusion and trafficking in the Als2-null mice. They analysed the uptake of epidermal growth factor (EGF) by fibroblasts isolated from these mice and showed that although the endocytosis of the EGF receptor *per se* does not require ALS2, the trafficking and fusion of EGF-positive endosomes/vesicles in mutant cells were significantly delayed, suggesting that ALS2 might regulate the efficiency of these events in the cell (Hadano et al. 2006) (figure 1).

Further evidence linking the intracellular membrane proteins to motor neuron disease has come from the identification of a missense mutation (P56S) in the vesicle-associated membrane protein/synaptobrevin-associated membrane protein B (VAPB) in patients with atypical amyotrophic lateral sclerosis (ALS8). This is an autosomal dominant adult onset disease characterised by fasciculation, cramps and postural tremor (Nishimura et al. 2004). Vesicle associated proteins such as VAPB are intracellular membrane proteins that associate with microtubules and are involved in membrane transport (figure

1). While the wild-type VAPB protein localises predominantly to the endoplasmic reticulum, the distribution of its P56S mutant form is dramatically disrupted leading to aggregate formation (Nishimura et al. 2004).

Motor proteins and MND

Kinesins

The superfamily of kinesin proteins (KIFs) is comprised of 14 families most of which form a long filamentous structure with the globular motor domain at one end and the cargo binding domain at the other end (reviewed by Miki et al (Miki et al. 2001)). The neuron specific member of the kinesin superfamily KIF5A has been implicated in motor neuron disease. Mutations, including a missense N256S substitution mutation, in KIF5A cause the autosomal dominant pure hereditary spastic paraplegia 10 (SPG10). Asparagine 256 is within a loop/helix motif of the motor domain of the protein (Reid et al. 2002). Mutations in this region have been shown to disrupt the microtubule binding of the motor domain leading to the failure of the microtubule-dependent stimulation of the ATPase activity of the kinesin motor (Song and Endow 1998). Subsequent screening of other SPG10 families have lead to the identification of other mutations including R280C, Y279C, and A361V in the same microtubule binding motif of the motor domain (Blair et al. 2006; Fichera et al. 2004; Lo Giudice et al. 2006). Evidence suggests that KIF5A participates in a heterotetrameric complex (Goldstein and Yang 2000). Thus the likely explanation for the autosomal dominant inheritance of SPG10 is a dominant-negative effect and/or haploinsufficiency. Both childhood and adult-onset cases have been reported in SPG10 families and it remains to see

whether the early-onset disease correlates with any possible defects in axon development. Although data from knockout KIF5A mice show that null KIF5A mutants die neonatally and that, compared with control littermates, the spinal cord motor neurons appear to have bigger nuclei and cell bodies (Xia et al. 2003).

Dynein

Cytoplasmic dynein consists of two homodimerised heavy chains and multiple accessory proteins (King 2000). The C-terminal portion of dynein provides the force for movement of dynein along microtubules. The N-terminal domain is responsible for heavy chain homodimerisation and binding of accessory proteins including intermediate, light intermediate, and light chains. Dynactin is another complex which interacts with cytoplasmic dynein through dynein intermediate chains and regulates its functions. In addition, it acts as an adaptor for binding to some of the cargo transported by dynein. Dynactin is composed of several subunits including $P150^{Glued}$ and dynamitin (P50) (Vallee et al. 2004).

Defects in cytoplasmic dynein have been shown to cause motor neuron degeneration in several mouse models. The *Legs at odd angles* (*Loa*) and *Cramping 1* (*Cra1*) mice, generated in two independent *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis programmes, manifest progressive locomotor disorders with autosomal patterns of inheritance (Hafezparast et al. 2003). Hafezparast et al showed that substitution mutations F580Y and Y1055C in the cytoplasmic dynein heavy chain in *Loa* and *Cra1* respectively impair

retrograde axonal transport leading to motor neuron degeneration. They also demonstrated that these mutations perturb neuronal development and migration but leave housekeeping functions of cytoplasmic dynein intact (Hafezparast et al. 2003). In addition, LaMonte et al have shown that the inhibition of dynein-mediated axonal transport, by postnatal over-expression of the dynactin component dynamitin, also results in motor neuron degeneration (LaMonte et al. 2002). Indeed, a G59S mutation in P150^{Glued} subunit of dynactin has been identified in families with slowly progressive autosomal dominant forms of motor neuron disease (Munch et al. 2004; Puls et al. 2003; Puls et al. 2005). Affected individuals with this mutation develop early adulthood vocal fold paralysis which causes breathing difficulty, progressive facial weakness, and weakness and atrophy in distal-limb muscles (Puls et al. 2003). Studies on the G59S mutation have revealed that the mutation is in a highly conserved domain which interacts directly with microtubules and the microtubule plus-end protein EB1 (Ligon et al. 2005). Subsequently Levy et al have provided evidence that the G59S mutation leads to decreased microtubule binding and enhanced dynein and dynactin aggregation. This dual effect of the mutation suggests that both toxic gain-of-function and loss of function may contribute to the degeneration of motor neurons in this disease (Levy et al. 2006).

SUPEROXIDE DISMUTASE 1 (SOD1) AND AXONAL TRANSPORT IN MND

ALS is the most common adult onset form of motor neuron diseases. It is a progressive disease that specifically targets the motor neurons in the spinal cord, brain stem, and cortex (Mulder et al., 1986; Rowland and Shneider,

2001). It was first described by Charcot in 1869 and has a worldwide incidence of 1-2 in 100,000 individuals with a lifetime risk factor of 1 in 2,000. The number of affected men is approximately 1.6 fold higher than that of women. ALS strikes mainly in midlife with an average age of onset between 50 and 60 years and it is characterised by selective and progressive degeneration of motor neurons leading to muscle weakness, wasting and spasticity and eventually death within one to five years of onset, mainly because of respiratory failure (Brown 1995; Bruijn et al. 2004; Cleveland and Rothstein 2001; Rowland and Shneider 2001; Shaw 1999). Early symptoms of ALS include fatigue and fasciculations followed by muscle weakness usually in the limbs. As a result, in the early stages of the disease, the ALS patients experience difficulty in coordination and in some cases involvement of the bulbar muscles leads to slowing of speech or difficulty in swallowing.

Protein aggregation and inclusion body formation are common features of the late onset neurodegenerative diseases such as ALS, Alzheimer's, Parkinson's, Huntington's and prion disease. The inclusion bodies in ALS consist of ubiquinated protein aggregates mainly seen in the spinal motor neurons and motor cortex (Proukakis et al. 2004). In addition, neurofilament accumulation is seen in the axons of motor neurons in ALS (reviewed by Bruijn et al (Bruijn et al. 2004)).

Approximately10% of ALS cases show familial inheritance that is usually autosomal dominant. Of these, 20–25% are mapped to the Cu, Zn-*SOD1* gene on chromosome 21 (Rosen et al., 1993) where over 100 individual

mutations have been identified, some of which retain complete enzymatic activity (Borchelt et al., 1994; Bowling et al., 1995; Gaudette et al., 2000). In the remaining 90% of patients, ALS is sporadic. The clinical manifestation of sporadic and familial ALS are very similar, although the age of onset is often lower for the familial form of the disease.

Despite intensive research the precise aetiology of the disease remains unclear, but transgenic mice over-expressing mutant forms of human SOD1 have been valuable tools in obtaining insights into the development of the disease and in highlighting the cellular functions that are the targets of the disease. These mice exhibit an ALS-like phenotype (reviewed by Shibata (Shibata 2001)). The presence of intracellular protein aggregates in motor neurons is a common feature of all reported cases of human ALS and the SOD1-transgenic mouse models. These aggregates stain positive for SOD1 and ubiquitin in the mouse models and all human SOD1-mediated familial ALS. Two main mechanisms have been proposed for the gain of toxicity of mutant SOD1 (reviewed by Bruin (Bruijn et al. 2004)). One suggests the involvement of Cu and Zn active sites, where a gain of aberrant chemistry in mutant SOD1 enzyme damages other proteins and subsequently leads to cell death. The second model implicates aggregates as the primary cause of motor neuron death. Several mechanisms have been proposed for the toxicities of these aggregates, including: aberrant chemistry, loss of protein function through co-aggregation with aggregates, depletion of protein folding chaperones, dysfunction of the proteasome overwhelmed with undegradable, misfolded protein, and inhibition of specific organelle function.

In addition, several studies have highlighted disruptions in axonal transport as major contributing factors in motor neuron cell death in ALS (Jablonka et al. 2004; Rao and Nixon 2003; Williamson and Cleveland 1999). Indeed, defects in axonal transport is one of the earliest pathologies observed in SOD1 mice (Kieran et al. 2005; Williamson and Cleveland 1999). Moreover, Ligon et al have shown that neurotracer transport from muscle to motor neurons is impaired in $SOD1^{G93A}$, and that there is an association of dynein with mutant SOD1 aggregates in the motor neurons of these mice (Ligon et al. 2005).

Furthermore, evidence suggest cell specific mechanisms involving motor neurons and microglia in disease initiation and progression in human ALS. Microglia are activated up on neuronal damage to initiate an inflammatory response, termed microgliosis, that affects neighbouring neurons and astrocytes. In ALS microgliosis is seen before motor neuron loss and it persists as the disease progresses. Biollée et al have recently reported their study on the roles of motor neurons and microglia in motor neuron disease (Boillee et al. 2006). They used a Cre-lox system to specifically reduce the expression of mutant SOD1 either in motor neurons or in the microglia and macrophages of mutant-SOD1 transgenic mice. In this study it was shown that lowering mutant SOD1 expression within microglia significantly extended the survival of the mice, mainly due to a slowing of the disease progression after onset. The disease onset and early progression, however, appeared to be related to the toxicity of the SOD1 within motor neurons and not microglia. These results therefore suggest that mutant SOD1 damage within motor

neurons underlies the onset and early progression of the disease, whereas progression to the late stages and complete paralysis in these mice is linked to the inflammatory response of microglia and the mutant toxicity within these cells (Boillee et al. 2006).

Interactions of dynein mutations and mutant SOD1

The implication of defective dynein in motor neuron degeneration in *Loa* and *Cra1* mice, and the evidence linking impaired axonal transport to the pathogenesis of motor neuron disease (Hafezparast *et al*, 2003, Science) prompted Kieran et al to study possible interactions between the *Loa* mutation and mutant SOD1 by crossing *Loa*/+ with SOD1^{G93A} transgenic mice. This cross produced double heterozygous (*Loa*/SOD1^{G93A}), as well as SOD1^{G93A}, *Loa*/+, and wild-type offspring. Parental SOD1^{G93A} and their SOD1^{G93A} pups from this cross showed a reduced life span of only 125 days, with disease endpoint defined as a loss of the righting reflex and 20% body weight. The end-stage for *Loa*/SOD1^{G93A} double mutants, however, was 160 days of age, an increase in lifespan of 28% (p=<0.001). Disease onset was also delayed in *Loa*/SOD1^{G93A} double mutants with a significant delay in the loss of body weight (Kieran et al. 2005). This increase in lifespan was not a result of loss of toxicity of the mutant SOD1 protein in the double mutants, as when *Loa*/SOD1^{G93A} mice were backcrossed to *Loa*/+ mice, their SOD1^{G93A} progeny had the same phenotype as parental SOD1^{G93A} mice. Nor was this increase in lifespan due to a lower amount of SOD1^{G93A} in the double mutants, as quantitative western blot analyses on brain and spinal cord samples showed that these mice had the same amount of SOD1^{G93A} protein as their SOD1^{G93A}

parents (Kieran et al. 2005). Intriguingly, analysis of the retrograde axonal transport revealed that defects in this pathway are already present in motor neurons of SOD1^{G93A} mice during embryonic development (Kieran et al. 2005).

Analyses of the nerve and muscle functions and histology of all the genotype cohorts established that the *Loa*/SOD1^{G93A} mice at 120 day of age (late stage for SOD1^{G93A}) show a wild type phenotype with regards to muscle force, motor unit, and motor neuron survival. In addition, kinetic analysis of retrograde axonal transport in motor neurons from wild-type, *Loa*/+, SOD1^{G93A}, and *Loa*/SOD1^{G93A} E13 embryos showed Loa/+ motor neurons displayed a speed profile very similar to that seen in wild-type, suggesting that two copies of a mutated dynein heavy chain are required to observe the dramatic alteration in retrograde transport previously detected in *Loa*/*Loa* homozygous mice (Hafezparast et al. 2003; Kieran et al. 2005). Surprisingly, the deficit in retrograde transport observed in SOD1^{G93A} motor neurons was completely rescued in *Loa*/SOD1^{G93A} (Kieran et al. 2005). Similarly, when *Cra1*/+ mice were crossed with SOD1^{G93A} the disease onset was delayed and the *Cra1*/SOD1^{G93A} double mutants lived longer than their SOD1^{G93A} littermates (Teuchert et al. 2006).

These results indicate that axonal transport defects play a critical role in motor neuron death in SOD1^{G93A} mice. Although the molecular mechanism by which dynein mutations induce amelioration of the disease in *Loa*/SOD1^{G93A} and *Cra1*/SOD1^{G93A} mice is not understood, it is clear that the specific impairment

of the neuronal function of cytoplasmic dynein rescues the defect observed in SOD1^{G93A} mice and produces a complete recovery of the axonal retrograde transport defect. This in turn may be responsible for the delay in disease progression and extension in lifespan observed in the double mutants. The improvement observed in the axonal retrograde transport in *Loa*/SOD1^{G93A} compared with SOD1^{G93A} mice might have occurred through restoration of the distribution of cargo within the motor neurons by rescuing the balance between anterograde and retrograde transport. Peripheral axotomy appears to be beneficial to survival of motor neurons in SOD1^{G93A} mice (Kong and Xu, 1999; . Comp. Neurol. 412:373–380). One therefore could argue that the amelioration of the disease in *Loa*/SOD1^{G93A} may be a result of an inhibitory effect of the *Loa* mutation in the transport of negative retrograde signals. Alternatively, it is possible that the dynein mutation results in abnormal intracellular transport, which in turn may change the localization and/or re-compartmentalization of mutant SOD1, for instance, interaction of mutant SOD1 with organelles such as mitochondria could be altered thus delaying cell death. This could be of special interest as the recruitment of mutant SOD1 to spinal cord mitochondria and sequestration of the anti-apoptotic protein Bcl-2 in the mitochondria have been suggested to be linked to mutant SOD1-mediated toxicity (Liu et al., 2004; Pasinelli et al., 2004). Equally, as the dynein mutation in *Loa* is autosomal dominant it is possible that the beneficial effects of this mutation in *Loa*/SOD1^{G93A} mice are the result of differential affinities of mutant SOD1 to wild-type and mutant dynein heavy chains with a higher affinity towards the *Loa* allele. The sequestration of the mutant dynein by mutant SOD1 would then allow normal dynein to assemble into functional

motors restoring the axonal retrograde transport. In support of this notion, mutant SOD1 has been reported to colocalize with dynein (Ligon et al, 2005). The possible association of mutant SOD1 with the wild type and mutant dynein complex is currently under investigation in the authors' laboratory.

CONCLUSIONS

Due to their size and physiology motor neurons are highly dependent on an efficient transport system for the movement of membrane organelles, protein complexes, and neuroprotective and signalling molecules along the axons. Genetic studies on motor neuron diseases in humans and mouse models have provided several lines of evidence linking motor neuron degeneration to impaired axonal transport. These studies implicate proteins involved in microtubule dynamics and regulation, vesicle-associated membrane proteins, and the microtubule associated motor proteins. In addition, there appears to be a complex interplay between different components of the axonal transport, which if compromised will lead to motor neuron dysfunction or death. Furthermore, it is apparent that there is an interaction between mutant SOD1 and axonal transport and that the modification of this interaction by partial inhibition of the dynein function as seen in *Loa* and *Cra1* heterozygotes may prove to be beneficial for delaying disease onset and extending the life span. Further studies are needed to identify other players involved in these interactions and to elucidate the detailed molecular mechanisms that lead to motor neuron disease or those that alleviate the disease.

REFERENCES

Baas PW, Deitch JS, Black MM, Banker GA. 1988. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci U S A 85(21):8335-8339.

Benashski SE, Harrison A, Patel-King RS, King SM. 1997. Dimerization of the highly conserved light chain shared by dynein and myosin V. J Biol Chem 272(33):20929-20935.

Blair MA, Ma S, Hedera P. 2006. Mutation in KIF5A can also cause adult-onset hereditary spastic paraplegia. Neurogenetics 7(1):47-50.

Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW. 2006. Onset and progression in inherited ALS determined by motor neurons and microglia. Science 312(5778):1389-1392.

Bommel H, Xie G, Rossoll W, Wiese S, Jablonka S, Boehm T, Sendtner M. 2002. Missense mutation in the tubulin-specific chaperone E (Tbce) gene in the mouse mutant progressive motor neuronopathy, a model of human motoneuron disease. J Cell Biol 159(4):563-569.

Brown RH. 1995. Superoxide dismutase in familial amyotrophic lateral sclerosis: models for gain of function. Curr Opin Neurobiol 5:841-846.

Bruijn LI, Miller TM, Cleveland DW. 2004. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci 27:723-749.

Burger J, Fonknechten N, Hoeltzenbein M, Neumann L, Bratanoff E, Hazan J, Reis A. 2000. Hereditary spastic paraplegia caused by mutations in the SPG4 gene. Eur J Hum Genet 8(10):771-776.

Cao TT, Chang W, Masters SE, Mooseker MS. 2004. Myosin-Va binds to and mechanochemically couples microtubules to actin filaments. Mol Biol Cell 15(1):151-161.

Cleveland DW, Rothstein JD. 2001. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2(11):806-819.

Cleveland DW, Sullivan KF. 1985. Molecular biology and genetics of tubulin. Annu Rev Biochem 54:331-365.

Duchen LW, Strich SJ. 1968. An hereditary motor neurone disease with progressive denervation of muscle in the mouse: the mutant 'wobbler'. Journal of neurology, neurosurgery, and psychiatry 31(6):535-542.

Escurat M, Djabali K, Gumpel M, Gros F, Portier MM. 1990. Differential expression of two neuronal intermediate-filament proteins, peripherin and the low-molecular-mass neurofilament protein (NF-L), during the development of the rat. J Neurosci 10(3):764-784.

Evans K, Keller C, Pavur K, Glasgow K, Conn B, Lauring B. 2006. Interaction of two hereditary spastic paraplegia gene products, spastin and atlastin, suggests a common pathway for axonal maintenance. Proceedings of the National Academy of Sciences of the United States of America 103(28):10666-10671.

Fichera M, Lo Giudice M, Falco M, Sturnio M, Amata S, Calabrese O, Bigoni S, Calzolari E, Neri M. 2004. Evidence of kinesin heavy chain (KIF5A) involvement in pure hereditary spastic paraplegia. Neurology 63(6):1108-1110.

Fliegner KH, Kaplan MP, Wood TL, Pintar JE, Liem RK. 1994. Expression of the gene for the neuronal intermediate filament protein alpha-internexin coincides

with the onset of neuronal differentiation in the developing rat nervous system. J Comp Neurol 342(2):161-173.
Goldstein LS, Yang Z. 2000. Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. Annu Rev Neurosci 23:39-71.
Hadano S, Benn SC, Kakuta S, Otomo A, Sudo K, Kunita R, Suzuki-Utsunomiya K, Mizumura H, Shefner JM, Cox GA, Iwakura Y, Brown RH, Jr., Ikeda JE. 2006. Mice deficient in the Rab5 guanine nucleotide exchange factor ALS2/alsin exhibit age-dependent neurological deficits and altered endosome trafficking. Human molecular genetics 15(2):233-250.
Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, Bowen S, Lalli G, Witherden AS, Hummerich H, Nicholson S, Morgan PJ, Oozageer R, Priestley JV, Averill S, King VR, Ball S, Peters J, Toda T, Yamamoto A, Hiraoka Y, Augustin M, Korthaus D, Wattler S, Wabnitz P, Dickneite C, Lampel S, Boehme F, Peraus G, Popp A, Rudelius M, Schlegel J, Fuchs H, Hrabe de Angelis M, Schiavo G, Shima DT, Russ AP, Stumm G, Martin JE, Fisher EM. 2003. Mutations in dynein link motor neuron degeneration to defects in retrograde transport. Science 300(5620):808-812.
Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Cattolico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig B, Weissenbach J. 1999. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nature genetics 23(3):296-303.
Hirokawa N. 1982. Cross-linker system between neurofilaments, microtubules, and membranous organelles in frog axons revealed by the quick-freeze, deep-etching method. J Cell Biol 94(1):129-142.
Hirokawa N, Funakoshi T, Sato-Harada R, Kanai Y. 1996. Selective stabilization of tau in axons and microtubule-associated protein 2C in cell bodies and dendrites contributes to polarized localization of cytoskeletal proteins in mature neurons. J Cell Biol 132(4):667-679.
Hirokawa N, Takemura R. 2005. Molecular motors and mechanisms of directional transport in neurons. Nat Rev Neurosci 6(3):201-214.
Jablonka S, Wiese S, Sendtner M. 2004. Axonal defects in mouse models of motoneuron disease. J Neurobiol 58(2):272-286.
Joshi HC. 1993. Gamma-tubulin: the hub of cellular microtubule assemblies. Bioessays 15(10):637-643.
Kanai Y, Hirokawa N. 1995. Sorting mechanisms of tau and MAP2 in neurons: suppressed axonal transit of MAP2 and locally regulated microtubule binding. Neuron 14(2):421-432.
Kaplan MP, Chin SS, Fliegner KH, Liem RK. 1990. Alpha-internexin, a novel neuronal intermediate filament protein, precedes the low molecular weight neurofilament protein (NF-L) in the developing rat brain. J Neurosci 10(8):2735-2748.
Kieran D, Hafezparast M, Bohnert S, Dick JR, Martin J, Schiavo G, Fisher EM, Greensmith L. 2005. A mutation in dynein rescues axonal transport defects and extends the life span of ALS mice. J Cell Biol 169(4):561-567.
King SM. 2000. The dynein microtubule motor. Biochim Biophys Acta 1496(1):60-75.
LaMonte BH, Wallace KE, Holloway BA, Shelly SS, Ascano J, Tokito M, Van Winkle T, Howland DS, Holzbaur EL. 2002. Disruption of dynein/dynactin

inhibits axonal transport in motor neurons causing late-onset progressive degeneration. Neuron 34(5):715-727.
Levy JR, Sumner CJ, Caviston JP, Tokito MK, Ranganathan S, Ligon LA, Wallace KE, LaMonte BH, Harmison GG, Puls I, Fischbeck KH, Holzbaur EL. 2006. A motor neuron disease-associated mutation in p150Glued perturbs dynactin function and induces protein aggregation. J Cell Biol 172(5):733-745.
Ligon LA, Lamonte BH, Wallace KE, Weber N, Kalb RG, Holzbaur EL. 2005. Mutant superoxide dismutase disrupts cytoplasmic dynein in motor neurons. Neuroreport 16(6):533-536.
Lo Giudice M, Neri M, Falco M, Sturnio M, Calzolari E, Di Benedetto D, Fichera M. 2006. A missense mutation in the coiled-coil domain of the KIF5A gene and late-onset hereditary spastic paraplegia. Arch Neurol 63(2):284-287.
Martin N, Jaubert J, Gounon P, Salido E, Haase G, Szatanik M, Guenet JL. 2002. A missense mutation in Tbce causes progressive motor neuronopathy in mice. Nat Genet 32(3):443-447.
Miki H, Setou M, Kaneshiro K, Hirokawa N. 2001. All kinesin superfamily protein, KIF, genes in mouse and human. Proc Natl Acad Sci U S A 98(13):7004-7011.
Mitsumoto H, Ferut AL, Kurahashi K, McQuarrie IG. 1990. Impairment of retrograde axonal transport in wobbler mouse motor neuron disease. Muscle & nerve 13(2):121-126.
Mitsumoto H, Kurahashi K, Jacob JM, McQuarrie IG. 1993. Retardation of fast axonal transport in wobbler mice. Muscle & nerve 16(5):542-547.
Muglia M, Magariello A, Nicoletti G, Patitucci A, Gabriele AL, Conforti FL, Mazzei R, Caracciolo M, Ardito B, Lastilla M, Tedeschi G, Quattrone A. 2002. Further evidence that SPG3A gene mutations cause autosomal dominant hereditary spastic paraplegia. Ann Neurol 51(6):794-795.
Munch C, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, Prudlo J, Peraus G, Hanemann CO, Stumm G, Ludolph AC. 2004. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. Neurology 63(4):724-726.
Nicholson SJ, Witherden AS, Hafezparast M, Martin JE, Fisher EM. 2000. Mice, the motor system, and human motor neuron pathology. Mamm Genome 11(12):1041-1052.
Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D, Kok F, Oliveira JR, Gillingwater T, Webb J, Skehel P, Zatz M. 2004. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. American journal of human genetics 75(5):822-831.
Okabe S, Hirokawa N. 1989. Rapid turnover of microtubule-associated protein MAP2 in the axon revealed by microinjection of biotinylated MAP2 into cultured neurons. Proc Natl Acad Sci U S A 86(11):4127-4131.
Otomo A, Hadano S, Okada T, Mizumura H, Kunita R, Nishijima H, Showguchi-Miyata J, Yanagisawa Y, Kohiki E, Suga E, Yasuda M, Osuga H, Nishimoto T, Narumiya S, Ikeda JE. 2003. ALS2, a novel guanine nucleotide exchange factor for the small GTPase Rab5, is implicated in endosomal dynamics. Human molecular genetics 12(14):1671-1687.
Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM. 2006. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). Neurology.

Parysek LM, Goldman RD. 1988. Distribution of a novel 57 kDa intermediate filament (IF) protein in the nervous system. J Neurosci 8(2):555-563.
Proukakis C, Cross H, Patel H, Patton MA, Valentine A, Crosby AH. 2004. Troyer syndrome revisited. A clinical and radiological study of a complicated hereditary spastic paraplegia. J Neurol 251(9):1105-1110.
Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, Mann E, Floeter MK, Bidus K, Drayna D, Oh SJ, Brown RH, Jr., Ludlow CL, Fischbeck KH. 2003. Mutant dynactin in motor neuron disease. Nat Genet 33(4):455-456.
Puls I, Oh SJ, Sumner CJ, Wallace KE, Floeter MK, Mann EA, Kennedy WR, Wendelschafer-Crabb G, Vortmeyer A, Powers R, Finnegan K, Holzbaur EL, Fischbeck KH, Ludlow CL. 2005. Distal spinal and bulbar muscular atrophy caused by dynactin mutation. Ann Neurol 57(5):687-694.
Quenneville NR, Chao TY, McCaffery JM, Conibear E. 2006. Domains within the GARP subunit Vps54 confer separate functions in complex assembly and early endosome recognition. Mol Biol Cell 17(4):1859-1870.
Rao MV, Nixon RA. 2003. Defective neurofilament transport in mouse models of amyotrophic lateral sclerosis: a review. Neurochem Res 28(7):1041-1047.
Reid E. 2003. Science in motion: common molecular pathological themes emerge in the hereditary spastic paraplegias. Journal of medical genetics 40(2):81-86.
Reid E, Connell J, Edwards TL, Duley S, Brown SE, Sanderson CM. 2005. The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B. Human molecular genetics 14(1):19-38.
Reid E, Kloos M, Ashley-Koch A, Hughes L, Bevan S, Svenson IK, Graham FL, Gaskell PC, Dearlove A, Pericak-Vance MA, Rubinsztein DC, Marchuk DA. 2002. A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). American journal of human genetics 71(5):1189-1194.
Ross JL, Wallace K, Shuman H, Goldman YE, Holzbaur EL. 2006. Processive bidirectional motion of dynein-dynactin complexes in vitro. Nat Cell Biol 8(6):562-570.
Rowland LP, Shneider NA. 2001. Amyotrophic lateral sclerosis. N Engl J Med 344:1688-1600.
Sanderson CM, Connell JW, Edwards TL, Bright NA, Duley S, Thompson A, Luzio JP, Reid E. 2006. Spastin and atlastin, two proteins mutated in autosomal-dominant hereditary spastic paraplegia, are binding partners. Human molecular genetics 15(2):307-318.
Schmitt-John T, Drepper C, Mussmann A, Hahn P, Kuhlmann M, Thiel C, Hafner M, Lengeling A, Heimann P, Jones JM, Meisler MH, Jockusch H. 2005. Mutation of Vps54 causes motor neuron disease and defective spermiogenesis in the wobbler mouse. Nat Genet 37(11):1213-1215.
Shaw PJ. 1999. Motor neurone disease. Bmj 318(7191):1118-1121.
Shibata N. 2001. Transgenic mouse model for familial amyotrophic lateral sclerosis with superoxide dismutase-1 mutation. Neuropathology 21(1):82-92.
Siegel GJ. 1999. Basic neurochemistry : molecular, cellular and medical aspects. Philadelphia: Lippincott-Raven Publishers. xxi, 1183 p. p.
Song H, Endow SA. 1998. Decoupling of nucleotide- and microtubule-binding sites in a kinesin mutant. Nature 396(6711):587-590.
Teuchert M, Fischer D, Schwalenstoecker B, Habisch HJ, Bockers TM, Ludolph AC. 2006. A dynein mutation attenuates motor neuron degeneration in SOD1(G93A) mice. Exp Neurol 198(1):271-274.

Troy CM, Brown K, Greene LA, Shelanski ML. 1990. Ontogeny of the neuronal intermediate filament protein, peripherin, in the mouse embryo. Neuroscience 36(1):217-237.
Vallee RB, Williams JC, Varma D, Barnhart LE. 2004. Dynein: An ancient motor protein involved in multiple modes of transport. J Neurobiol 58(2):189-200.
Weber KL, Sokac AM, Berg JS, Cheney RE, Bement WM. 2004. A microtubule-binding myosin required for nuclear anchoring and spindle assembly. Nature 431(7006):325-329.
Williamson TL, Cleveland DW. 1999. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat Neurosci 2(1):50-56.
Wood JD, Landers JA, Bingley M, McDermott CJ, Thomas-McArthur V, Gleadall LJ, Shaw PJ, Cunliffe VT. 2006. The microtubule-severing protein Spastin is essential for axon outgrowth in the zebrafish embryo. Human molecular genetics 15(18):2763-2771.
Xia CH, Roberts EA, Her LS, Liu X, Williams DS, Cleveland DW, Goldstein LS. 2003. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. J Cell Biol 161(1):55-66.
Zhao C, Takita J, Tanaka Y, Setou M, Nakagawa T, Takeda S, Yang HW, Terada S, Nakata T, Takei Y, Saito M, Tsuji S, Hayashi Y, Hirokawa N. 2001. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. Cell 105(5):587-597.

Fig. 1. Disease mechanisms in MNDs in relation to the axonal transport system.

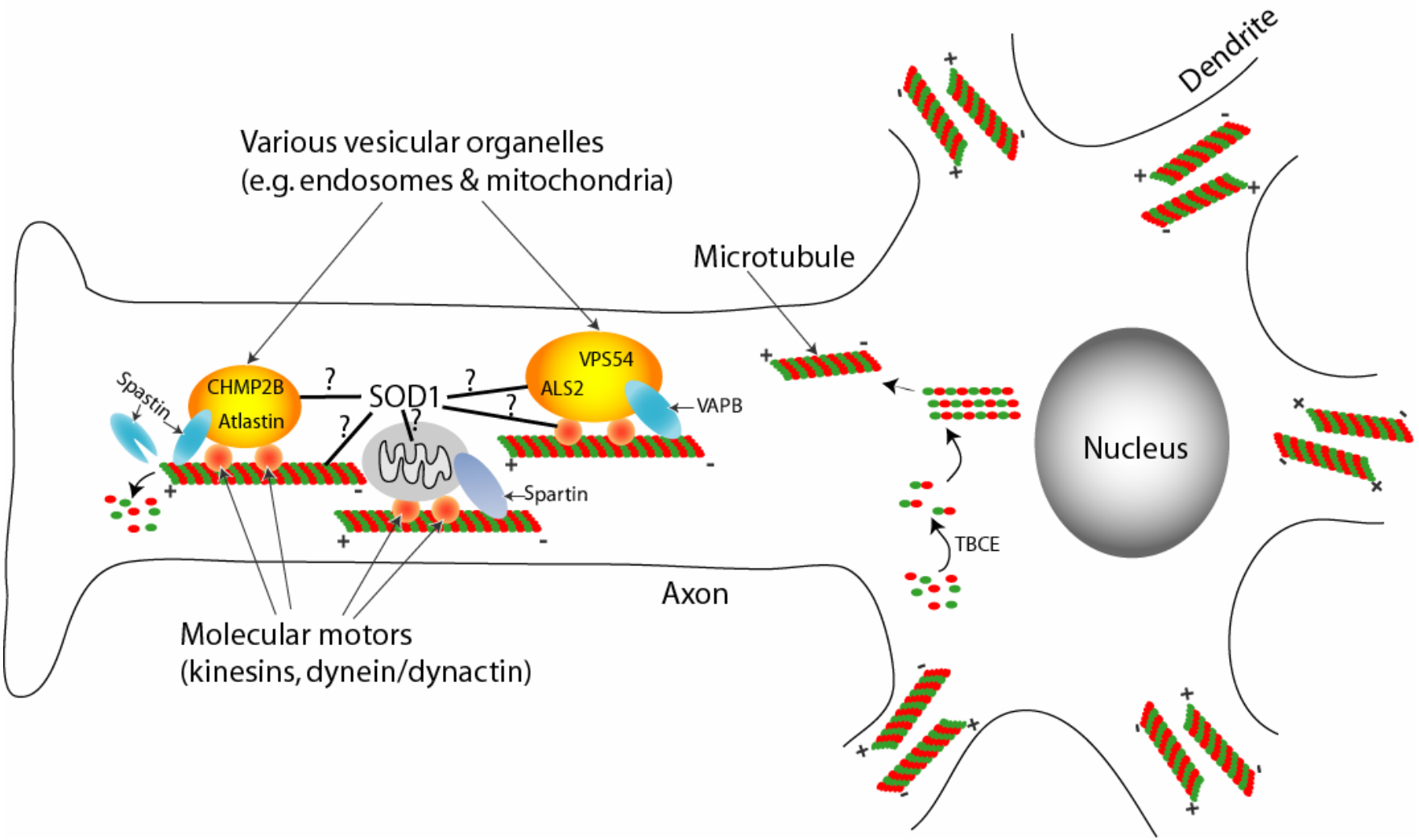

Table I: Components of axonal transport that are mutated in motor neuron diseases

Gene/protein	Mutation	Disorder	References
Microtubule-dynamic -associated proteins:			
Tubulin-specific chaperone E (TBCE)	W524G	Progressive motor neuropathy (*pmn*) – Mouse model	Bommel et al. 2002; Martin et al. 2002
Spastin	Various mutations (missense mutations – e.g. S362C, C448Y & R499C; nonsense mutations – e.g. 1520delT; splice-site mutations)	Spastic paraplegia 4 (SPG4)	Hazan et al. 1999
Vesicle-trafficking -associated proteins:			
Atlastin	R239C, S259Y and H258R.	Spastic paraplegia 3 (SPG3)	Zhao et al. 2001
Amyotrophic lateral sclerosis 2 (ALS2)	Deletion mutations (e.g. 261delA) and single-base substitutions (e.g. C873T)	Type 3 autosomal recessive amyotrophic lateral sclerosis (AR-ALS)	Hadano et al. 2001 & 2006
Vacuolar-vesicular protein sorting factor-54 (VPS54)	L967Q	Wobbler (*wr*)– Mouse model	Duchen and Strich 1968; Mitsumoto et al. 1990 & 1993; Schmitt-John et al. 2005; Quenneville et al. 2006
Vesicle-associated membrane protein/synaptobrevin-associated membrane protein B (VAPB)	P56S	Spinal muscular atrophy (SMA) and Atypical amyotrophic lateral sclerosis (ALS8)	Nishimura et al. 2004
Spartin	1110delA founder frameshift mutation	Troyer syndrome (SPG20)	Patel et al. 2002; Proukakis et al. 2004
CHMP2B (charged multivesicular body protein 2B, also known as chromatin-modifying protein 2B)	I29V and Q206H	ALS spectrum disorders	Parkinson et al. 2006
Motor and motor -associated proteins:			
Cytoplasmic dynein heavy chain (DNCHC1)	F580Y (*Loa*), Y1055C (*Cra1*)	Legs at odd angles (*Loa*) and Cramping 1 (*Cra1*) – Mouse models	Hafezparast et al. 2003
Kinesin (KIF5A)	N256S, Y279C, R280C, A361V	Hereditary spastic paraplegia 10 (SPG10)	Reid et al. 2002; Fichera et al. 2004; Blair et al. 2006
Dynactin $p150^{glued}$	G59S	Progressive lower motor neuron disease	LaMont et al. 2002; Munch et al. 2003; Puls et al. 2003 & 2005
Other Genes/Proteins:			
Cu, Zn-Superoxide dismutase1 (Cu, Zn-SOD1)	Over 100 various mutations	Familial ALS in humans and mouse models	Rosen et al. 1993